ously showed a higher degree of thymic involution but oral administration was also quite effective. The fact that the total amount of orally administered cortisone was ineffective when given as a single dose suggests that a cumulative effect may be involved in the physiological action of cortisone.

Summary. (1) Thymic weight loss in 14 hours offers an accurate quantitative endpoint by means of which to measure the action of cortisone when administered by different routes. (2) Under conditions of our experiment, cortisone was much more effective when administered by subcutaneous route as compared with the oral or intrasplenic route. The subcutaneous:oral ratio is about 1:15. (3) Sensitivity of adrenalectomized mice to the thymolytic action of cortisone did not differ from that of intact animals. (4) Administration of small doses of cortisone, which caused thymic involution when given by subcutaneous route, failed to do so when given by intrasplenic or oral route. This suggests that inactivation by the liver, previously found in *in vitro* experiments, also plays a role *in vivo*. If the dose administered through the portal routes is large, sufficient amounts escape inactivation to produce a maximal effect upon the thymus.

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Effect of ACTH and Cortisone on Phagocytosis. (18899)

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The experiments to be reported represent an attempt to determine the effect of adrenocorticotrophic hormone (ACTH) or cortisone treatment on the activity of the polymorphonuclear neutrophilic leukocyte in man. This study was prompted by the occasional progression of infection, possibly due to impaired phagocytic function, in patients undergoing treatment with these agents. For example, bacteremia with extensive abscess formation, without the usual systemic and local reactions, has been observed here and elsewhere. Some of these occurrences are cited by Mote(1). The general plan of the investigation was to use whole defibrinated blood from patients before and after treatment as a source of leukocytes, combined with virulent type I pneumococci and anti-type I pneumococcus rabbit serum. The organisms and antiserum were presumably unvarying, and any differences in phagocytic activity could be attributed to an effect, either direct or indirect, of the drugs on the leukocytes.

Methods and materials. The patients under examination were suffering from a variety of conditions which are indicated in Table I. A blood sample was obtained, defibrinated with glass beads, and tested before treatment was begun, and again after 4 to 21 days of therapy. Blood was taken at the same time each day; the patient was still under treatment when the last specimen was taken. The type I pneumococcus was maintained in cooked meat medium in the refrigerator for the duration of the experiment and no mouse or other passage of the organism took place. Ten to 12 hour cultures were made from this stock in 2% Bacto-tryptose, 0.5% glucose, 0.5% NaCl, 10% horse serum broth. The organisms were then neutralized to about pH 7.6, sedimented and suspended in 0.85% saline. The turbidity of the suspension in a 13 mm inside diameter cuvette was adjusted to 35% transmission at a wave-length of 620 Å in a Coleman Junior spectrophotometer. The antiserum stored in the frozen state was all of the same lot, small portions being thawed and diluted in 0.85% saline as needed.

Screw-cap vials, 6 x 1 cm, were loaded with 0.5 ml of blood, 0.1 ml of organism suspension and 0.1 ml of varying 2-fold dilutions of anti-

1. Proc. 1st Clin. ACTH Conf., edited by John R. Mote, Blakiston Co., 1950.

TABLE I. Effect of ACTH or Cortisone on Phagocytosis.

Patient	Dilution of antiserum 1/80 Pre‡ P.I.*	% †	Post§ P.I.	%	1/160 Pre P.I.	%	Post P.I.	%	1/320 Pre P.I.	%	Post P.I.	%	Saline control Pre P.I.	%	Post P.I.	%
1. Rheumatoid arthritis cortisone 50 mg t.i.d. × 9 days	—	‖	—		4.1	82	1.7	58	.98	52	.9	16	0	0	.06	6
2. Diffuse collagenic disease ACTH 15 mg q 6 hr × 7 days. 25 mg t.i.d. × 3 days	9.12	100	7.9	100	5.72	98	1.08	50	1.6	54	.48	26	.28	14	.08	8
3. Rheumatoid arthritis cortisone 50 mg t.i.d. × 7 days	—		9.10	92	2.56	72	1.08	32	.28	20	.40	22	.14	8	.06	6
4. Rheumatic fever cortisone 300 mg stat 100 mg daily × 9 days	7.04	94	4.9	70	1.62	50	1.08	36	.18	10	.08	8	0	0	.02	1
5. Bronchial asthma cortisone 100 mg daily × 10 days ACTH 100 mg daily × 7 days	7.3	92	—		1.3	47	7.2	100	.22	12	5.2	100	.06	6	4.6	100
6. Psychoneurosis cortisone 100 mg daily × 5 days	13.4	100	9.28	99	4.2	68	1.88	56	.32	18	.48	18	.18	12	.04	1
7. Rheumatoid arthritis ACTH 25 mg q.i.d. × 7 days	6.56	98	8.6	88	1.76	64	.70	30	.68	28	.20	8	0	0	0	0
8. Psychoneurosis ACTH 25 mg t.i.d. × 5 days	23	98	4.9	82	.67	55	.9	36	.56	32	.06	6	.14	6	.02	2
9. Infectious hepatitis cortisone 50 mg b.i.d. × 5 days	2.8	60	.58	24	.72	26	.48	20	.20	12	.24	10	.04	4	.04	4
10. Rheumatoid arthritis ACTH 25 mg t.i.d. × 7 days	8.54	96	.18	16					.16	12	0	0	.04	4	0	0

* P.I. = Avg No. of organisms per cell (phagocytic index). † % = % of neutrophiles taking up organisms. ‡ Pre = Blood taken before treatment. § Post = Blood taken after treatment. ‖ — = Too numerous to count.

serum starting at a dilution of 1:10. A saline control, without antiserum, was included. A 3 mm stainless steel ball was placed in the tube which was closed with a double layer of parafilm as a gasket under the screw cap. The tubes were rotated at 2 RPM end-over-end at 37°C for 20 minutes. Wright's stained films were prepared and the number of organisms taken up by 50 neutrophiles, as well as the number of participating neutrophiles was determined.

Results. It is apparent (Table I) that leukocytic activity is lower in the blood sample taken after treatment with ACTH or cortisone than in that obtained before treatment. In some cases this decrease is marked and in others it appears to be relatively slight. In only one instance (patient 5) did the activity increase; this appeared in the saline control also, and was shown to be due to the development of specific antibody during treatment, since the patient's serum undiluted or diluted 1:4, added to normal non-immune whole blood stimulated marked phagocytosis; no clinical manifestation of infection was noted. In another instance not shown in the table in which no pre-treatment blood was obtained, the patient developed multiple staphylococcal abscesses from which the causative organism was isolated. It was found that abundant phagocytosis of this organism took place in the patient's own blood. Since no comparable pre-treatment figures are available, it is obviously impossible to make any statement concerning the development of antibody, or the relative decrease in the activity of the phagocyte. Incidentally, this individual was receiving penicillin when the infection appeared, the organism was penicillin-susceptible, and regression of the abscesses occurred promptly when ACTH therapy was withdrawn.

Whether the consistently observed depression of phagocytic activity is of sufficient magnitude to account for increased susceptibility to infection is not certain although it might be at least a contributory factor. Alteration in complement content could explain this effect, but it has been shown(2) that, if anything,

2. Pitner, G., Smith, L. C., and Calwell, C. A., *Bact. Proc.*, 1951, p88.

complement levels rise during ACTH treatment.

Summary. In 9 of 10 patients under treatment with ACTH or cortisone, the phagocytic activity of the neutrophilic leukocyte decreased. In the remaining case an increase was observed which was shown to be due to the development of specific antibody during treatment.

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A Study of the Transfer of Serum Proteins into Tissue Injured by Tourniquet.* (18900)

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During the past 20 years, a large quantity of evidence has accumulated indicating that in traumatic shock there is a displacement of fluid from the blood into the tissue of the injured area. Blalock(1) reported that the loss of circulating volume could be accounted for by the gain in weight of the traumatized area. This observation has been supported by the work of Nickerson(2), Ashworth, Jester, and Lloyd(3), Swingle *et al.*(4), and others, and it has been concluded by most investigators in this field that the loss of plasma is the principal cause of the condition known as shock. Inasmuch as plasma is a complex substance consisting of several protein components as well as electrolytes and water, it is necessary to determine specifically the transfer of each of these substances in order to understand the role they play in the syndrome of shock.

This paper presents analytical data on the nature of the proteins found in injured and uninjured tissue and compares them with those found in serum.

General procedure. Partial ischemia in one hind leg of each of 6 anesthetized dogs was produced by binding with a tight tourniquet. The animals were anesthetized by intravenous injection of nembutal (0.6 cc of a 6% solution per kg body weight). The tourniquets were made tight by first wrapping about 10 feet of elastic bandage high up on the thigh, then a double loop of insulated wire which was drawn tight by twisting with pliers. This procedure produces neither complete nor reproducible degrees of ischemia, the variation being partially dependent on the size of the animal used. The bandage was left for 4 to 5 hours (Table I) and during the entire experiment small quantities of nembutal were administered when needed to keep the animal in deep anesthesia. Sometime during this period a relatively large quantity of the blue dye, T-1824, was injected via jugular vein. This was done for blood volume determinations and also to see whether dye-tagged albumin could be traced into the tissues. Rawson(5) has shown that 8 to 14 molecules of dye are bound by one molecule of albumin. In these experiments the number of moles of dye used was much less than the number of moles of albumin in the blood so that the albumin should be far from saturated and the binding relatively complete.

At about 1½ hours after release of the tourniquets the animals were exsanguinated and in one case (Dog No. 2) both femoral

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2. Nickerson, J. L., *Am. J. Physiol.*, 1945, v144, 429.

3. Ashworth, C. T., Jester, A. W., and Lloyd, G. E., *Am. J. Physiol.*, 1944, v141, 571.

4. Swingle, W. W., Remington, J. W., Kleinberg, W., Drill, V. A., and Eversole, W. J., *Am. J. Physiol*, 1942, v138, 156.

5. Rawson, R. A., *Am. J. Physiol.*, 1943, v138, 708.